

SUITABILITY OF VARIOUS BUFFERS FOR GENOMIC DNA EXTRACTION OF PINEAPPLE (*Ananas comosus*) VAR. MD2

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Abstract: Methods for DNA extraction are of paramount importance to obtain high yield and high purity nucleic acids for molecular characterisation downstream. However, there is no specific extraction protocol developed for *Ananas comosus* var. MD2. Here, we compare the efficiency of five selected DNA extraction buffers which are extensively used for plant deoxyribonucleic acid (DNA) extraction with absence usage of phenol. The suitability of the extraction buffers was assessed based on both DNA yield and its quality. In this study, DNA extracts were quantified using ultraviolet (UV) spectrophotometry, spectrophotometric profiles and gel electrophoresis. Since interfering background substances are not visible in gel electrophoresis, examining PCR products of the crude DNA is recommended. To summarise, all the buffers yielded sufficient DNA of an approximate 50-200 µg from 1 g leaf tissue for downstream applications with different quality level. Out of five extraction methods, two give high yield and high-quality genomic DNA using Dellaporta-based method (213.5 µg/g) and Doyle & Doyle-based method (172.5 µg/g). Among these extraction methods, the exclusion of detergents in extraction buffer served as the best extraction buffer for MD2 genomic DNA extraction. Also, from an economic point of view, the extraction buffer is cheaper compared to commercial DNA extraction kits.

Keywords: DNA extraction, *Ananas comosus* var. MD2, UV spectrophotometry, spectrophotometric profile, PCR.

Introduction

Molecular techniques and other downstream applications related to these techniques such as cloning, gene library construction and polymerase chain reaction (PCR) are powerful tools in the science field. The success of these downstream methods is strongly dependent on DNA extraction efficiency and the quality of the isolated DNA (Rana *et al.*, 2019; Rawat *et al.*, 2016). In addition, easy handling, time-saving and affordable extraction protocols are among the requirements in choosing extraction methods. In general, achieving high-quality DNA is essential for ensuring consistency in all subsequent steps in the analytical measurement process.

The isolation of high-quality DNA from plant tissue samples is relatively difficult compared to genomic DNA isolation from animal tissues (Abdel-Latif & Osman, 2017; Anderson

et al., 2018) due to the excessive presence of secondary metabolites and high polysaccharides content (El-Ashram *et al.*, 2016). Contaminants such as polysaccharides resulted in handling difficulties (Abdel-Latif & Osman, 2017) and also found to cause anomalous re-association kinetic (Murray & Thompson, 1980). Thus, the presence of these compounds can severely affect the quality and quantity of the isolated DNA. Acid phenol is commonly used to eliminate protein from genomic DNA, however, phenol is omitted in this analysis as this reagent is highly toxic to both mankind and the environment (El-Ashram *et al.*, 2016; Rivero *et al.*, 2006). In addition, Li *et al.* (2017) reported that phenol could significantly interfere with UV absorbance ratio quantitation by absorbing light in the range of 260 to 280 nm. Besides that, working with phenol is labour-extensive as chloroform extraction is needed to remove phenol from the isolated DNA (Kasem *et al.*, 2008). Apart from